Genetic relationship of interspecies for eight birch species

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Abstract: Genetic relationships of eight species of genus *Betula* were evaluated using ISSR marks. A total of 236 loci were generated from 17 ISSR primers. Percentage of polymorphic bands (PPB) varied from 5.93 to 19.92. The highest and the lowest level of genetic differentiation were detected in *B. ovalifolia* and *B. maximowicziana* Regel respectively. In these eight species, genetic diversity of birch (H_T) was 24.38 %, and the genetic variation (G_{ST}) interspecies was accounting for 79.36% of total genetic variation. According to the cluster results of genetic distance, the eight species were classified into three groups as *B. davurica*, *B. ovalifolia*, *B. platyphylla* and *B. pendula* for one group; *B. schmidtii*, *B. costata* and *B. ermanii Cham.* var. *communis* for one group, and *B. maximowicziana* Regel for another group. The result of cluster is consistent with traditional morphological classification.

Key words: Birch; Inter simple sequence repeat; Genetic relationship, Interspecies
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Introduction

Analysis of interspecific relationship is one of the important fields in molecular systematics. Different molecular marks will produce particular band patterns in different populations, or specific bands of genus. Thus DNA molecular marks have been widely used in the specific classification and genetic relationship analysis of interspecies (Zou 2001).

Inter Simple Sequence Repeat (ISSR) is a technology that uses SSR primers to amplify regions between simple repetitive DNA sequences. Since it was first reported by Zietkiewicz *et al.* (1994) in 1994, this technology with great repeatability has been used in classification and systematic comparison of species, deducing evolutionary relationship of species and identification of varieties and it also serves as a tool for genetic mapping (Tsumura 1996; Wolfe 1998; Culley 2001).

In this paper, we analyzed the genetic relationship of interspecies for eight birch species by ISSR technology and reconstituted the dendrogram of testing birch species in molecular level. This study will provide scientific evidence for promoting the development of hybridization and sufficiently exploring gene source of birch. The birch species used in this experiment are *Betula platyphylla*, *B. davurica*, *B. costata*, *B. ermanii Cham.*var. *communis*, *B. ovalifolia*, *B. schmidtii*, *B. maximowicziana* Regel, and *B. pendul.*

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Materials and methods

Plant materials

Birch seeds collected from China and Japan (Table 1) were sown and grown in the greenhouse of Tree Breeding Base of Northeast Forestry University, China. Fresh leaves from seedling were stored at -70℃ for molecular analysis.

Table 1. Resources of birch samples

Species	Resources
Betula costata	Heilongjiang, China
*B. ermanii Cham.*var. *communi*	Heilongjiang, China
B. platyphylla	Heilongjiang, China
B. schmidtii	Liaoning, China
B pendula	Foundation of Finnish Forest Tree Breeding
B. maximowicziana Regel	Hokkaido Regional breed office in Japan
B. davurica	Hokkaido Regional breed office in Japan
B. ovalifolia	Hokkaido Regional breed office in Japan

Extraction of total DNA

Total DNA was extracted from frozen leaves by the procedure of CTAB protocol (Jiang 2001). The quality and quantity of total genomic DNA were detected by the UV/V1S Spectrometer Lambda Bio10 produced by PERKIN ELMER Company of USA.

Screen of ISSR primers and detection of amplified products by PCR

The sequences of ISSR primers were provided by Columbia University, Canada, which were fifty dinucleotide repeated sequences and synthesized by TaKaRa Company. Amplification was performed in Perkin-Elmer 9700 Thermal Cycler, according to the following RAPD reaction system of

B. platyphylla (Jiang 2001). Initial denaturation was for 5 min at 94 °C, followed by 30 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 51 °C, extension for 30 s at 72 °C and a final 7 min extension at 72 °C. The amplifying products were segregated in 1.5% agarose gels (contain EB 0.5 μg/ml). After electrophoresis, gels were put into UVP Gel Documentation Systems (GDS7600) for observing and taking photos. From the result, 17 primers were screened from fifty initial primers (Table 2).

Table 2. Sequences of selected ISSR primers

Primers	Sequences (5'-3')
807	$(AG)_8T$
811	$(GA)_8C$
812	$(GA)_8A$
814	$(CT)_8A$
815	$(CT)_8G$
821	$(GT)_8T$
822	$(TC)_8A$
823	$(TC)_8C$
826	$(AC)_8C$
827	$(AC)_8G$
828	$(TG)_8A$
834	$(AG)_8YT$
836	$(AG)_8YA$
844	$(CT)_8RC$
845	$(CT)_8RG$
852	$(TC)_8RA$
853	$(TC)_8RT$

Notes: R stands for A and T. Y stands for G and C

Statistical analysis

Each band in electrophoresis profile represents a pair of complementary combined loci of primer and annealing DNA template, also as a molecular mark. By the corresponding place of DNA ladders in gel, we can easily estimate the sizes of the target fragments. Number 1 indicated the presence of a single band in certain place and number 0 indicated the absence of a single band. Finally, we put the 0/1 matrix into computer.

Using Popgen32 (Francis *et al.* 1977) software to process data, we obtained the Shannon diversity index and Nei index of ISSR-PCR products, studied the genetic variation distribution of interspecies and intraspecies, calculated genetic distance of interspecies, and reconstructed the dendrogram of the species.

Results and analysis

Percentage of polymorphic bands

Percentage of polymorphic bands (PPB) is one of the indexes to measure genetic variation standard, and 92 individuals of 8 species in *Betula* were analyzed by ISSR-PCR. The selected primers totally generated 236 loci, whose size is between 250 bp and 2 500 bp (Fig. 1). The range of PPB is from 5.93% to 19.92%, and the species of the highest PPB is *B. ovalifolia* with the highest level of genetic variation, and that of the lowest PPB is *B. maximowicziana* Regel with the lowest genetic variation level (Table 3).

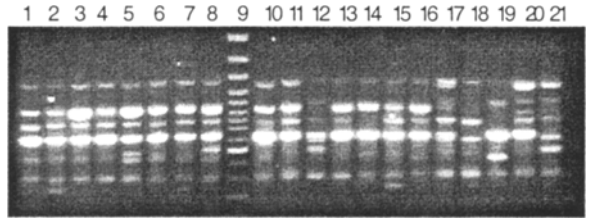

Fig.1 Profile of amplifying products by primer 811

Note: 1-8, 10, 11--*B. platyphylla*; 12-21-- *B. pendula*; 9-- DNA ladder (MBI), the sizes from top to bottom are 3 Kb, 2 Kb, 1.5 Kb, 1.2 Kb, 1 Kb, 0.9 Kb, 0.8 Kb, 0.7 Kb, 0.6 Kb, 0.5 Kb, 0.4 Kb, 0.3 Kb, 0.2 Kb, 0.1 Kb in order.

Estimating *Betula* genetic variation by Shannon index and Nei index

Shannon index of birch species was calculated by Popgen32 software (see Table 3). The corresponding order of the species from the smallest to the biggest variation were: *B. maximowicziana* Regel<*B. davurica*<*B. pendula*<*B. costata*<*B. platyphylla*<*B. schmidti*<*B. ermanii Cham.*var. *communis*<*B. ovalifolia*. Nei index varied from 0.0211 to 0.0792, and the order of species was: *B. maximowicziana* Regel<*B. davurica*<*B. pendula*<*B. platyphylla*<*B. costata*<*B. schmidti*<*B. ermanii Cham.*var. *communis*<*B. ovalifolia* (Table 3). From the results, the two indexes used to evaluate birch genetic diversity were coincident, and were basically identical with the results of the percentage of polymorphic bands (PPB).

Infraspecific and interspecific genetic differentiation

Genetic differentiation was estimated using Nei's parameters. Total genetic diversity (H_T) for polymorphic loci was 24.38 % in tested species, in which diversity within species (H_S) was 5.03%, and genetic diversity among species (D_{ST}) was 19.35%. The differentiation of among species was accounting for 79.36% of total genetic diversity (G_{ST}) (G_{ST}= D_{ST}/H_T; Nei 1973, 1977). Thus the results showed that the genetic variation of interspecies was very high.

Analysis of genetic identity and genetic clustering

Using Popgen32 software, we analyzed the data of amplification products and gained the genetic identity and genetic distance of interspecies for birch species (Table 4). Birches in the test were clustered in a distance from 0.1108 to 0.3264. For this reason, we obtain the result of high genetic analogy degree among birch species, of which the most analogical species were *B. pendula* and *B. platyphylla*, and the most dissimilar species were *B. platyphylla* and *B. maximowicziana* Regel. Eight species were classified into three groups by genetic cluster in this experiment (Fig.2):

1) *B. davurica*, *B. ovalifolia*, *B. platyphylla*, *B. pendula*.

2) *B. schmidtii*, *B. costata*, *B. ermanii* Cham. var. *communi*

3) *B. maximowicziana* Regel

Of all the species, three pairs showed high analogy: *B. platyphylla* and *B. pendula* whose genetic distance was 0.1108, is the most similar pair, followed by the pair *B. costata* and *B. ermanii* Cham. var. *communis* with genetic ditance of 0.2141, and the third pair is *B. davurica* and *B. ovalifolia*, with the genetic distance of 0.2354.

Table 3. Comparison of genetic variation in birch

Species	No. of individuals	Total of ISSR bands	No. of polymorphic bands	PPB /%	Nei index	Shannon index
B. maximowicziana Regel	15	236	14	5.93	0.0211	0.0314
B. Davurica.	15	236	26	11.02	0.0360	0.0539
B. pendula	10	236	23	9.75	0.0389	0.0566
B. costata	10	236	36	15.25	0.0559	0.0828
B. platyphylla	10	236	39	16.53	0.0550	0.0830
B. schmidtii	10	236	38	16.10	0.0579	0.0854
*B. ermanii Cham.*var. *communis*	7	236	38	16.10	0.0585	0.0871
B. ovalifolia	15	236	47	19.92	0.0792	0.1154

Table 4. Genetic identity and genetic distance interspecies birch species

Species	*B. davurica*	*B. schmidtii*	*B. costata*	*B. platyphylla*	*B. pendula*	*B. ovalifolia*	*B. maximowiczziana* Regel	*B. ermanii Cham.* var. *communis*
B. davurica.	****	0.7534	0.7250	0.7970	0.7711	0.7902	0.7278	0.7346
B. dchmidtii	0.2831	****	0.7914	0.7753	0.7509	0.7564	0.7350	0.7667
B. costata	0.3216	0.2339	****	0.7591	0.7505	0.7723	0.7709	0.8073
B. platyphylla,	0.2269	0.2545	0.2756	****	0.8951	0.7886	0.7215	0.7789
B. pendula	0.2599	0.2864	0.2870	0.1108	****	0.7782	0.7423	0.7787
B. ovalifolia	0.2354	0.2791	0.2584	0.2375	0.2507	****	0.7879	0.7950
B. maximowicziana Regel	0.3178	0.3079	0.2602	0.3264	0.2980	0.2384	****	0.7712
*B. ermanii Cham.*var. *communis*	0.3084	0.2657	0.2141	0.2499	0.2501	0.2294	0.2598	****

Notes: Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

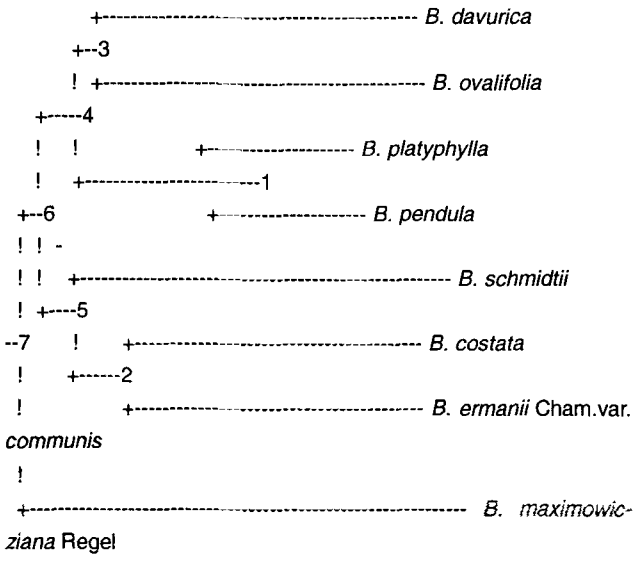

Fig. 2 Dendrogram interspecies birch species based on the genetic distances generated by Popgen32 software

Discussion

Genetic diversity of *Betula*

Betula includes more than 30 species, which widespread distributes in almost every province of China. The complexity of genetic variation was due to the variable climate and complicated geography condition in birch habitant. In this experiment, three indexes were used to analyze the degree of genetic variation of testing species, and all the results were parallel. Of all the testing species, *B. ovalifolia* presented the highest degree of genetic differentiation, followed by *B. ermanii* Cham.var. *communis*, *B. schmidti*, *B. platyphylla* and *B. costata*, and the lowest one was *B. maximowicziana* Regel. Generally, the maintenance of high levels of genetic differentiation may represent different adaptability (G Ledyard 1963). Hence, great potential selection capability exists in high genetic differentiated species. This is useful to explore and to make a full use of this gene resource in the future.

Consistence between molecular relationship reconstruction and traditional classification

With the rapid development of molecular biological technology, classical observation method for relationship

analysis had been replaced by relationship reconstruction based on polymorphism of nucleic acid that forms the basic matter of creature inheritance.

Birch species involved in this research belonged to the following five Subsect of Sect. *Betula* in traditional classification, Subsect. Betula: *B. Platyphylla, B. pendula*; Subsect. Dahuricae Regel: *B. maximowicziana regel*; Subsect. Costatae Regel: *B. ermanii* Cham.var. *communis*, *B. costata*; Subsect. Fruiticosa Regel: *B. ovalifolia*; Subsect. Chinenses Schneid: *B. schmidtii.* Relationship reconstructed in DNA level was coherent with traditional classification, that is to say, birch species in the same Subsect clustered into one group in genetic analysis.

In this experiment, *B. davurica* and *B. maximowicziana* Regel seeds collected in Hokkaido region in Japan were considered as two kinds of species because of the great difference in term of morphologic traits and wood-quality, meanwhile, and the result of ISSR also indicated that there are far distance in genetic analogy, relatively. Though *B. davurica* and *B. maximowicziana* Regel were regarded as one group according to the reports (The Chinese Academy of Science 1979), what is the relationship of the two in DNA level? Further study should be done.

Leading specific hybridization of birch

Hybridization among wood species has been paid more attention by breeding experts for a long time. Stern and Naoei ITAHANA had studied specific hybridization of birch, but the technical result cannot be extended to large-scale application (Klaus 1974; Naoei 1997). The related studies showed that hybridization is relatively easy in closed related species. Although they are in far distance or in different flowering phase, only pollens of these two closed relatives are artificially controlled, it is probably to yield intercrosses. The analysis of genetic relationship in DNA level demonstrated in this investigation will provide scientific evidence in order to promote hybridization breeding in birch species.

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